

## **Blood Products Advisory Committee Meeting**

**March 13, 2003**

**Topic: Validation of nucleic acid tests (NAT) to screen blood and plasma donors for acute infection with West Nile virus (WNV)**

**Issue: FDA seeks advice from the Blood Product Advisory Committee on 1) the design of scientific studies needed to validate NAT and possibly IgM for WNV as blood donor screening tests; 2) whether available data on clearance of viruses in the manufacture of plasma derivatives are a sufficient basis to obviate screening of Source Plasma donations; and 3) whether strategies to limit WNV screening to particular locations and times are appropriate.**

### **I. Background Information**

#### *A. West Nile virus and transmission through blood:*

The West Nile Virus (WNV) is a mosquito-borne flavivirus that primarily infects birds, and occasionally horses and humans. In humans, about 80% of infections are asymptomatic, and in about 20% a mild febrile illness develops, but in about 1 in 150 infections a meningitis or encephalitis occurs. Advanced age is by far the most significant risk factor for severe neurologic disease. The viremic period is transient, and can occur up to 2 weeks prior to symptoms and last up to a month from the initiation of the infection. Virus titer in blood is low compared to other transmissible viruses ( $\sim 1-5 \times 10^3$  copies/ml), but in encephalitis patients can be as high as  $2.5 \times 10^6$  copies/mL. Viremia resolves rapidly after seroconversion to WNV IgM antibody, and it appears that IgM can persist for a long time, in some cases up to a year. So far no chronic stage of WNV infection has been reported.

In 2002 the total number of WNV cases reported was 4008, with 263 deaths and 2741 cases of West Nile meningoencephalitis. Thirty-nine states, including DC, have reported WNV infection. CDC estimated a theoretical risk of 1.8-2.7 infections per 10,000 donations in the 1999 Queens, New York, epidemic. However, in the 2002 epidemic estimations were as high as 16/10,000, with a mean of 6-8/10,000 in heavily endemic regions. Blood transmission of WNV has been confirmed in the US outbreak last year. However, the true magnitude of the risk of WNV from transfusion is unknown. From August 28, 2002 to January 3, 2003, CDC reported 61 possible transfusion-transmitted cases. Twenty-one are confirmed, 19 are not transfusion-related, and 21 are still under investigation.

#### *B. FDA's Actions to Date*

On August 17, 2002, prior to reports of transmission by blood, alert notices were posted on FDA's website urging vigilance in excluding symptomatic donors. On October 3, 2002, FDA stated its interest in facilitating development of donor screening and supplemental tests. FDA initiatives included the distribution of an FDA Guidance document on donor and

product management, facilitating development of screening and supplemental tests, and identification of needs for additional research in regard to WNV.

FDA held a scientific workshop on WNV on November 4 and 5, 2002. The workshop covered a very wide range of topics including; a review of methodologies suitable for donor screening; a review of proposed studies on prevalence in donors and the status of these studies; industry and FDA perspectives on developing WNV assays; strategies aimed at inactivation of WNV in plasma derivatives. The details of the workshop were summarized for the Committee at the December 2002, BPAC meeting.

## **II. Regulatory Pathway for WNV Blood Donor Tests**

### *A. Approval Mechanism:*

As with other tests used in the manufacture of blood and blood components, WNV donor screening and supplemental assays would be reviewed as biologic products under the PHS act. This requires the submission of an Investigational New Drug (IND) application with a description of clinical trial plans and, eventually, pre-market filing of a Biologic License Application (BLA). In assessing assays used to test blood donors, FDA considers the following criteria ; clinical and analytical sensitivity and specificity; chemistry, manufacturing and controls; reproducibility, proficiency; stability; instrumentation and software.

### *B. Clinical Study Design:*

FDA recognizes the need to implement testing in a timely manner and would, therefore, allow large-scale studies and widespread use of tests under the Investigational New Drug (IND) application if necessary. This approach would permit blood centers to introduce testing while evaluating test performance in the intended use setting. In the case of WNV infection, all the known transmissions by blood transfusion have occurred in the acute, viremic, phase indicating that this phase of infection would be the ideal target for intervention. Therefore, it is expected that implementation of sensitive NAT assays would be the most useful strategy for interdicting potentially infectious units. It is also anticipated that most testing methods would use pooled samples rather than individual donations, at least for the present time, due to practical constraints. However, concerns exist about the sensitivity limits of current NAT assays for WNV and the impact of pooling on detection of specimens with low levels of virus. These concerns raise the question of whether IgM antibody assays should also play a role in donor screening for WNV since it is known that viremia and detectable IgM can coexist in the early phase of infection. Lack of data on WNV transmission by donations that are minipool NAT negative and IgM positive suggest that it may be useful to explore, in clinical studies, whether a combination of NAT and IgM would provide added assurance of blood safety in regard to WNV. In the text below, FDA will outline some potential considerations for clinical validation of NAT and IgM assays intended for donor screening during studies under IND.

As part of clinical validation, FDA has traditionally required that a test be evaluated for clinical specificity, clinical sensitivity and reproducibility and that these studies be conducted at a minimum of 3 clinical sites.

For validation of the clinical specificity of a NAT assay for WNV, it is FDA's current thinking that the test should be evaluated in a low risk population from areas of low prevalence. In general FDA considers a sample size of 10,000 tests (pooled or individual) to be adequate. Data on clinical specificity could be obtained by identification of negative cases during prospective studies conducted at various clinical sites under IND, and retrospective studies of repository specimens identified in clinical and donor settings during previous epidemics. For investigational NAT assays, the true viral marker negative status of potentially NAT false-positive specimens could be established by additional testing with an alternate NAT (using primers from a different region of the viral genome) and follow-up testing, including lack of IgM seroconversion. In the case of tests for IgM, FDA's current thinking is that confirmation of true-negative results could be obtained by using a NAT and second IgM test, with discordant results resolved by neutralization assays. Because the sensitivity of current minipool and IgM assays for WNV have not yet been well established, during clinical trials, it is worth considering whether all donations should be tested using both NAT and IgM assays.

Clinical sensitivity for a donor screening indication for a NAT or IgM assay could be determined by testing samples from existing repositories provided sample integrity has been preserved by appropriate storage. Testing of repository specimens collected during epidemics of previous years, including transfusion and WNV illness related specimens from donor and community settings, will likely be a critical component of sensitivity studies for validation of WNV assays, since the extent of future epidemics and frequency of new infections remains unknown at this time. Identification of positive cases during prospective IND studies conducted in blood bank and community settings could provide additional data for clinical sensitivity of either a NAT or IgM assay. For a diagnostic indication, specimens from cases of WNV illness in community and clinical settings could be tested. The viral marker positive status of specimens could be established by use of an alternate NAT and follow-up testing including IgM seroconversion. In general, FDA believes that the true outcome of testing for clinical specificity and sensitivity should be determined by additional testing including the use of an alternate method and follow-up.

Since there are no existing reference or licensed assays for testing, FDA is considering some additional approaches for test validation. It is FDA's view that testing of all reactive samples identified in clinical studies by all manufacturers seeking licensure of WNV tests would be useful in determining whether candidate investigational tests have equivalent sensitivity. This approach would require that adequate volumes of specimens be stored under appropriate conditions and that blood and plasma centers cooperate to make them available for such testing. Additionally, FDA has initiated efforts to assemble an in-house qualification panel composed of well-characterized and pedigreed specimens to further establish the relative sensitivity of NAT and IgM assays. FDA believes that this combination of approaches would facilitate a comprehensive evaluation of NAT and IgM assays for WNV. It should be pointed out that FDA's current analytical sensitivity standard for WNV NAT assays is 100 copies/ml for the individual donation. This standard may be revised as tests become more sensitive, or as additional data are obtained on the levels of viremia and infectivity in future studies.

FDA believes that investigational tests for WNV should be evaluated for their ability to detect various strains of the virus. If the test is designed to detect all members of the Flavivirus Genus, it would be useful to demonstrate the ability to detect all members of the virus family with equal sensitivity. These studies could be conducted with repository specimens from previous epidemics and well-characterized panels.

In regard to WNV testing of Source Plasma donations, it should be noted that no cases of transmission through plasma-derived products have been demonstrated so far. Also, preliminary experimental data suggest comparability of WNV clearance to the clearance of flavi-model viruses commonly used in validation studies, although the level of assurance of these methods has not been established. Despite these observations, FDA remains concerned about the potential for WNV transmission by these products in the future. Therefore, FDA's current thinking is that tests for WNV screening might also be validated for use with Source Plasma donations. For this purpose, studies similar to those outlined for screening of whole blood collections could also be conducted in the Source Plasma setting.

Finally, FDA's current thinking is that reproducibility studies should be conducted at a minimum of 3 sites. The studies would include testing of a panel of well-characterized specimens including low positive specimens by multiple operators at different sites on different days using different operators and production lots.

### *C. Unit and Donor Management:*

FDA has recently published guidance for donor deferral, product quarantine and retrieval related to post-donation illnesses in the donor or WNV infection in the recipient (<http://www.fda.gov/cber/guidelines.htm>). When tests become available, consensus algorithms will need to be developed across testing platforms for unit and donor management. During the clinical trial FDA will consider strategies for donor and unit management in order to gather data regarding which of these strategies would provide maximum assurance of safety against WNV transmission by blood. This approach may be necessary since the performance characteristics of investigational tests and viral dynamics in WNV infection of humans are not well established. However, at this time, FDA is considering recommending some interim approaches for unit and donor management. When the IND studies are completed, FDA may revisit these strategies if necessary.

i. Unit management: Similar to algorithms currently in place for HCV and HIV NAT, when a reactive NAT result for WNV is obtained on a master pool, subsequent testing would be performed to identify the individual unit that is positive and the basis for the reactive result on the pool. The test result on the individual donation is considered to indicate the infectious status of the donation. If one or more reactive donations were identified upon individual donation testing, all non-reactive units could be released (if donations are deemed otherwise suitable for release). The reactive donation(s) would be quarantined, and destroyed as a general practice. They might, however, be used for research or as reagents for in-vitro diagnostic products, provided they are labeled for these purposes. During clinical trials a validated, alternate NAT method could be used to confirm the results of the investigational test on the individual donation. Reactive results obtained with the

investigational test could be confirmed by testing of a follow-up sample. The sample could be tested using the investigational NAT, and validated IgM and alternate NAT assay. A positive IgM test result would confirm the reactive NAT result obtained on the index donation. This additional testing would help validate the investigational test and establish its performance characteristics with regard to sensitivity and specificity. If a multiplex flavivirus assay were used, reactive results could be discriminated with regard to WNV infection.

If a master pool were reactive and all individual donations were non-reactive, we would consider it appropriate that a fresh specimen from each of the index donations be tested using the original NAT and the alternate NAT method. If reactive results were obtained on further testing, the donor could be notified of deferral. During the clinical trial, a reactive NAT result on the individual donation could be further confirmed by a second, alternate NAT method, which uses a different set of primers, in addition to IgM seroconversion.

ii. Donor management: It is FDA's opinion that if a donor's sample were to test positive on the individual donation as defined above and were either positive or negative on the IgM assay, it would be prudent for the donor to be temporarily deferred, notified of test results and counseled appropriately. FDA suggests a deferral period of 28 days consistent with the longest known duration of the viremic period. During the clinical trial, the donor would be enrolled in follow-up studies to document IgM seroconversion with a suitable serologic test. The donor could be re-tested prior to 28 days to confirm results obtained on the index donation. If negative NAT results were obtained, the donor could be reinstated after the 28 day period. If NAT results were positive, the donor would be deferred for an additional 28 days. If on follow-up testing prior to 28 days, NAT results were negative and IgM results were positive, the donor would continue to remain deferred until 28 days after the positive NAT results on the index donation. If testing was not performed during the 28 days, the donor could be automatically reinstated after this deferral period.

### **III. Testing Source Plasma Donors and Clearance of WNV from Plasma-Derived Products**

The viral safety of plasma-derived products with regard to the relevant pathogens (HIV, HBV, HCV) has been assured by a combination of blood donor deferral, testing of donations, and by the inclusion of viral clearance (inactivation and/or removal) steps in the manufacturing processes. In the face of potential risk from WNV infection, FDA has taken a conservative approach to ensure blood product safety. As such, efforts to develop suitable donor screening, testing and re-evaluation of viral clearance strategies for flaviviruses have been ongoing. FDA has recommended precautionary deferral of blood and plasma donors who may be infected with WNV. The most recent FDA guidance recommending such donor deferrals was published in October 2002 (<http://www.fda.gov/cber/guidelines.htm>).

Accumulating experimental data, and the absence of reported transmission of WNV by plasma-derived products suggest that common viral clearance methodologies, currently used in the manufacture of plasma derivatives, are also effective in the clearance of WNV. As such, plasma derivatives have a higher safety margin with regard to potential WNV infection than the components of Whole Blood (packed Red Blood Cells, Platelets, and Fresh Frozen Plasma), which are not virally inactivated. However, testing of the starting materials for the

presence of WNV, when such tests become available, for the purpose of eliminating positive units and reducing the viral load in the manufacturing pool, would ensure excess clearance capacity of any given manufacturing process, and would provide a higher degree of assurance with regard to the safety of plasma-derived products.

Different manufacturing conditions could substantially influence the clearance capacity of a given inactivation/removal step. Therefore, claims for removal or inactivation of pathogens from plasma derivatives have been based upon convincing viral validation data that are product and process-specific. Viruses used in these validation studies are a selection of enveloped and non-enveloped DNA and RNA viruses, which are relevant viruses or specific models for such viruses. Whenever technically feasible, the actual virus of concern should be used in viral validation studies, e.g., human immunodeficiency virus. However, if a virus of concern cannot be cultured, e.g., hepatitis C virus (HCV), then specific model viruses are used in the validation studies. Specific model viruses are selected based on taxonomical and physicochemical similarities to the virus of concern. With regard to flaviviruses, different specific model viruses (BVDV, SINV and TBEV) have been used to validate the effectiveness of common clearance methodologies in clearing the most relevant flavivirus to date, HCV. The results from these validation studies, and those obtained using HCV in animal model experiments, have demonstrated significant and comparable clearance level for these viruses. Limited, but growing experimental data indicates that these viral clearance steps are equally effective against WNV. However, further product process and WNV specific validation studies are needed, to provide further assurance on the robustness and reproducibility of these steps with regard to WNV clearance.

#### **IV. Implementation of WNV Donor Testing**

Several overlapping issues are related to the implementation of WNV donor testing. These include triggers for WNV testing and also blood supply management, in the event the test is not available at the time of an epidemic. Other implementation issues are worthy of consideration, such as seasonal and geographical characteristics of future WNV infection outbreaks, the possible occurrence of other related flaviviruses and the feasibility of using minipool vs individual NAT testing.

Currently, FDA is of the opinion that if WNV donor screening assays are available, but are not yet licensed when the epidemic begins (a human case of WNV infection has been diagnosed), universal donor screening for WNV should be implemented in the U.S., under IND if necessary and to the extent feasible.

Surveillance data obtained by the Centers for Disease Control and Prevention during the 2002 WNV epidemic indicate that reported human WNV meningoencephalitis and WNV fever cases provide a reliable indication of early human WNV exposure in a narrowly-defined geographic area, such as a US county. Such data may be useful to target the testing of donated blood if test supplies are limited.

FDA is also considering additional protective measures, including the identification and temporary deferral of donors who report the occurrence of fever for a limited period

immediately before blood donation (or product recall and quarantine if reported immediately following donation) during a time of community WNV exposure.

Consideration of a range of protective measures will be necessary to ensure the availability of a safe blood supply, given the uncertainties associated with a possible WNV outbreak in 2003. These advantages and disadvantages of potential strategies are under active discussion by FDA, together with other government Agencies and the blood organizations. Effective donor and product management plans will be particularly critical if WNV assays are not available at the beginning of an epidemic, or are only partially available.

## **V. Questions for the Committee:**

- 1. Please comment on FDA's proposed criteria for validation of WNV NAT and IgM assays for donor screening.**
- 2. Do the Committee members agree that product and process-specific clearance of the WNV agent (as opposed only to marker viruses) should be demonstrated in order to adequately assure the safety of plasma derivatives?**
- 3. Do the Committee members agree that screening of all plasma for fractionation for WNV would add a safety margin in the manufacture of plasma derivatives?**
- 4. Please comment on the scientific validity of possible strategies to limit WNV screening to particular locations and times depending on epidemic surveillance information and test availability .**

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